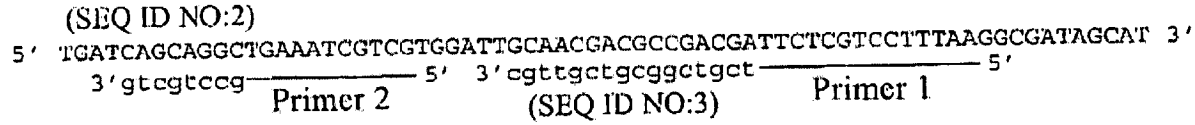
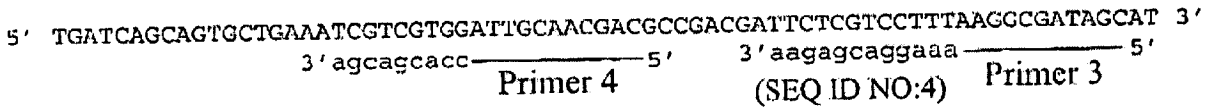
Target DNA Detection and Quantification

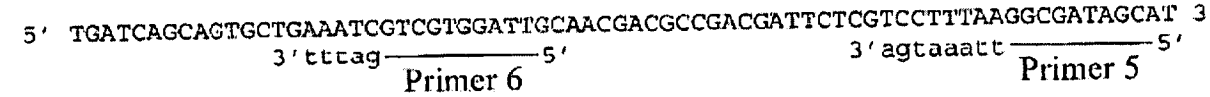
(1) Extension with dTTP, dGTP, dCTP, either with ddATP or without dATP



(2) Extension with dATP, dGTP, dCTP, either with ddTTP or without dTTP



(3) Extension with dATP, dGTP, dTTP, either with ddCTP or without dCTP



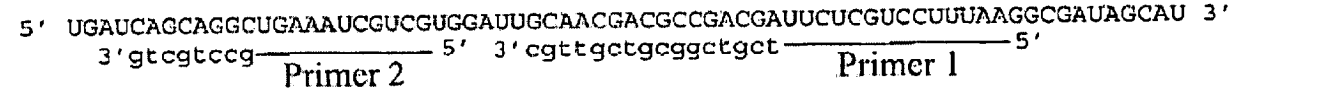
Line represents primer sequence. Small letters a, g, c, and t are bases extended from the primer, some of which are labeled with a detectable marker. u and t are used interchangeably.

Figure 1

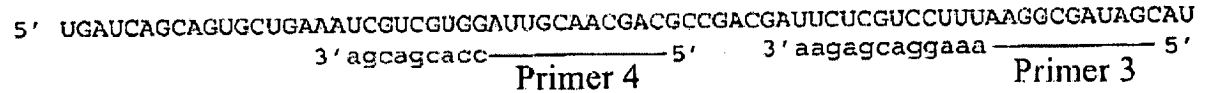
Target RNA Detection and Quantification

(1) Extension with dTTP, dGTP, dCTP, either with ddATP or without dATP

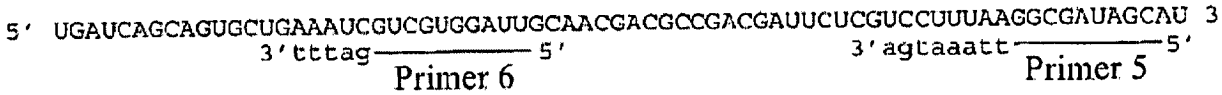
(SEQ ID NO:5)



(2) Extension with dATP, dGTP, dCTP, either with ddTTP or without dTTP



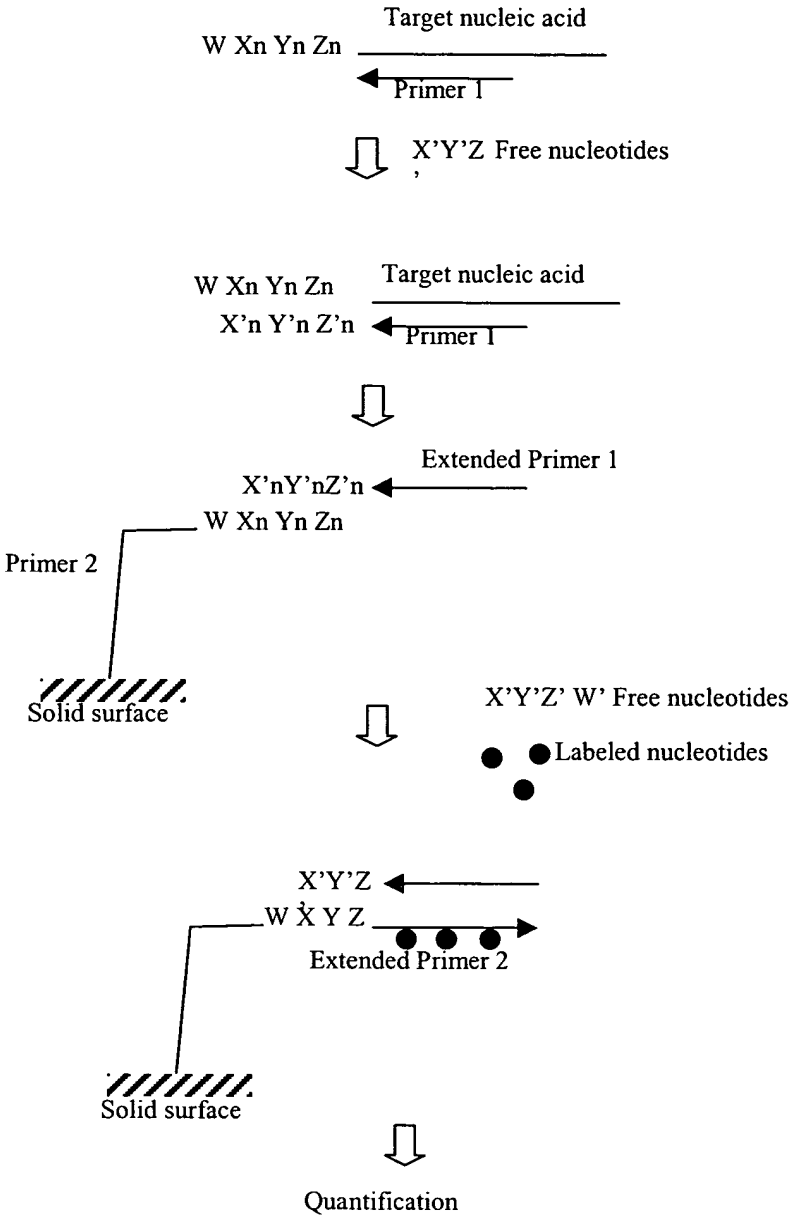
(3) Extension with dTTP, dGTP, dATP, either with ddCTP or without dCTP



Line represents primer sequence. Small letters a, g, c, and t are bases extended from the primer, some of which are labeled with a detectable marker. u and t are used interchangeably.

Figure 2

Figure 3



X =1st type of nucleotide	X'=nucleotide complementary to X
Y=2nd type of nucleotide	Y'=nucleotide complementary to Y
Z=3rd type of nucleotide	Z'=nucleotide complementary to Z
W=4th type of nucleotide	W'=nucleotide complementary to W
n>1	

Labeled nucleotide could be A, G, C or T

Figure 4

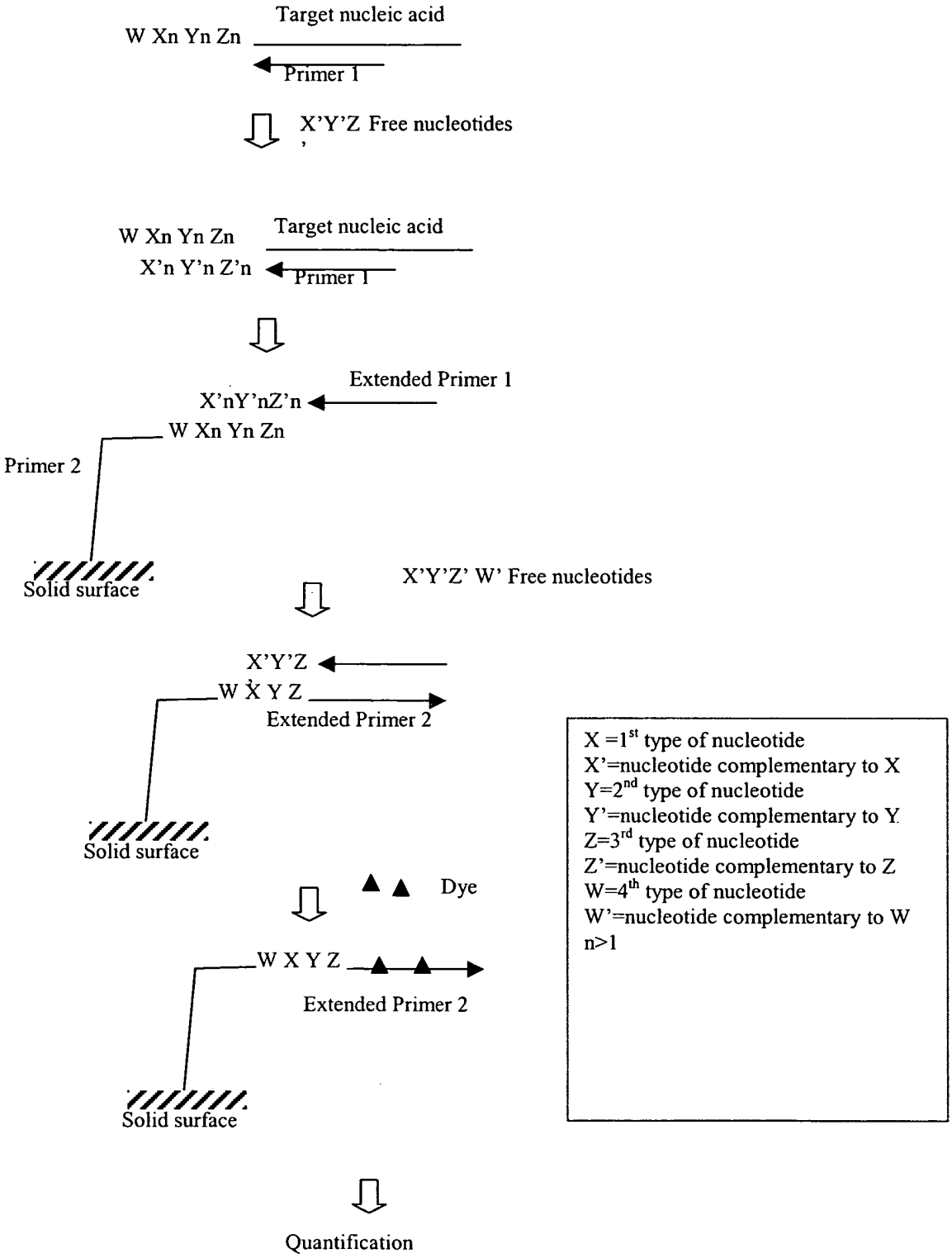

W Xn Yn Zn
Target nucleic acid
Primer 1
X'Y'Z Free nucleotides
W Xn Yn Zn
X'n Y'n Z'n
Target nucleic acid
Primer 1
Extended Primer 1
X'nY'nZ'n
W Xn Yn Zn
Primer 2
Solid surface
X'Y'Z' W' Free nucleotides
X'Y'Z
W X Y Z
Extended Primer 2
Solid surface
Dye
W X Y Z
Extended Primer 2
Solid surface
Quantification
X =1st type of nucleotide
X'=nucleotide complementary to X
Y=2nd type of nucleotide
Y'=nucleotide complementary to Y
Z=3rd type of nucleotide
Z'=nucleotide complementary to Z
W=4th type of nucleotide
W'=nucleotide complementary to W
n>1

Figure 5

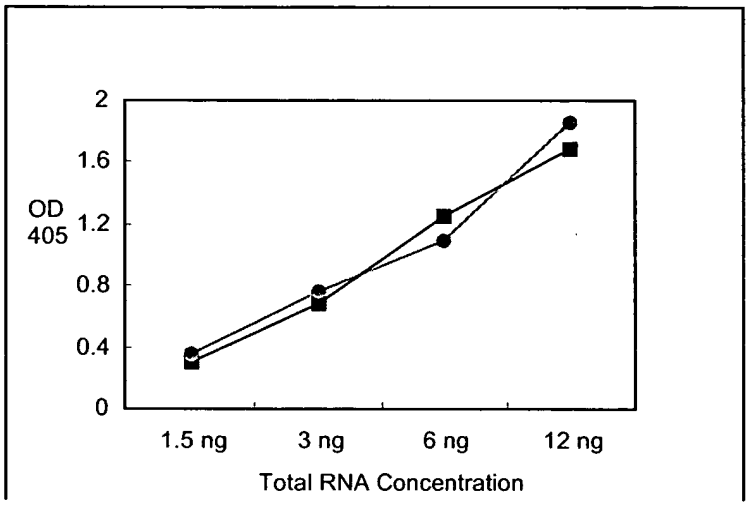
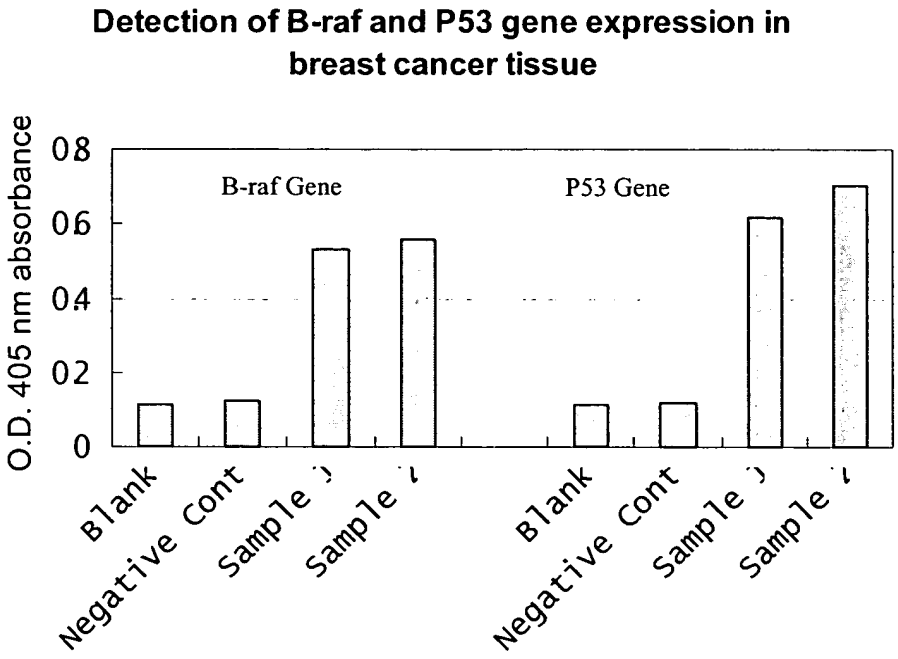

2
1.6
OD
405
1.2
0.8
0.4
0
1.5 ng
3 ng
6 ng
12 ng
Total RNA Concentration

Figure 6


Detection of B-raf and P53 gene expression in breast cancer tissue
O.D. 405 nm absorbance
08
06
04
02
0
B-raf Gene
P53 Gene
Blank
Negative Cont
Sample j
Sample i
Blank
Negative Cont
Sample j
Sample i